THE INFLUENCE OF CENTRIFUGAL FORCE ON THE BILATERAL DETERMINATION OF THE SPIRALLY-CLEAVING EGG OF URECHIS

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Introduction

There is evidence from the experiments of several workers, particularly Runnström (1926), Lindahl (1932), and Pease (1938), that the location of the plane of bilateral symmetry of various echinoderm eggs can be influenced by centrifugal force. It is of considerable interest, then, to determine whether this can be done with a spirally cleaving egg. The observations on cell lineage have led investigators to believe that, in general, in such eggs the position of the first cleavage plane determines the plane of bilateral symmetry. Further, it has been found by Just (1912), Morgan and Tyler (1930), Tyler (1931), and others that in such eggs the sperm entrance point is related to the first cleavage. However, Hörstadius (1937) has shown by vital staining that in the *Cerebratulus* egg the first cleavage may bear any relation to the bilateral axis. Furthermore, he concludes from the development of partial embryos that there is no evidence of bilateral determination at the two- or four-cell stage such as was indicated in the echinoderm egg by the experiments of Hörstadius and Wolsky (1936).

Eggs of the spirally cleaving type have been centrifuged by various workers including Boveri (1910), Conklin (1910, 1916), Hogue (1910), Lillie, F. (1909*a* and *b*), Morgan (1910), von Parseval (1922), Schleip (1914), Tyler (1930) and Wilson (1929 and 1930). None, however, have reported any influence of the stratification on the position of the median plane of the embryo. In the early experiments the centrifugal forces employed were relatively low. Also counts of the various types of embryos resulting were not made, it having been considered at best sufficient to demonstrate that embryos might be obtained with the different layers in any region. Much of the work was entirely unconcerned with the possibility that centrifuging could influence the bilateral determination.

I have therefore examined these questions in the *Urechis caupo* (Fisher and MacGinitie) egg using the high centrifugal forces possible with an air turbine ultracentrifuge. The results show a definite relation between the axis of stratification and the plane of bilateral symmetry in the resulting embryo. It is concluded that the stratification of material substances induces the ventral side near the centripetal end.

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Material and Methods

The eggs of the echiuroid worm, *Urechis caupo* (Fisher and MacGinitie), were used in these experiments. A brief description of the cleavage and trochophore stages has been given by Tyler (1931). The development is very similar to that of *Thalassema mellita* (Conn.) of which Torrey (1903) has carefully worked out the cell lineage.

The eggs were subjected to varying high centrifugal forces in an air turbine ultracentrifuge for short periods of time. They were suspended in the centrifuge tubes in about six parts of isotonic sucrose and four parts of sea water. This mixture was found to very nearly equal the specific gravity of the eggs. In calculating the forces applied, the speed of the rotor was known and the radius measured to the approximate position of the eggs in the centrifuge tubes. The forces are listed to the nearest thousand gravities and are within 10 per cent of the calculated figure. The eggs were centrifuged both before and after fertilization. Except where noted in the results, the viability of the centrifuged eggs seemed to approximate that of the controls and there was no indication of a differential mortality. The hardest centrifuging did not activate the eggs.

To study the eggs and embryos they were fixed in strong Flemming's solution, in general for about twelve hours, and then were washed for another twelve hours in water. Temporary glycerine preparations were then made. The oil during centrifuging goes to the centripetal end and occupies almost one-quarter of the periphery of the egg. It is stained jet black by this treatment and retains this color a number of hours when the eggs or embryos are cleared directly in glycerine. The black stain of the oil is by no means permanent in the glycerine and the embryos must be examined within a few hours after clearing them. The diffusion of the oil is so slow in the egg that a very good boundary is maintained until the trochophore stage. This in no way seems to effect the viability, and this condensed oil mass was found in otherwise normal trochophores in all regions of the embryo. It does not block cleavage and the cells containing great excesses of oil do not seem by superficial inspection to be abnormally large.

The centrifuging makes four primary layers in the unfertilized egg. Most centripetal is the large "oil cap," occupying about a quarter of the circumference. There follows a broad layer making up more than half of the egg by volume and about a half of the circumference. This contains the germinal vesicle which comes to lie just below the oil cap. The layer is fairly clear and can be recognized as a double, if not sometimes a triple layer. The centrifugal quarter of the circumference is occupied by the third and fourth layers. The third contains the bulk of the yolk and occupies most of the space. The fourth layer is made up of coarse pigment granules.

In studying the trochophores only those were considered that were normal or nearly so. Aberrant types were found to be difficult or impossible to orient. The aberrations found among the trochophores of the centrifuged eggs resembled those found in the control larvae and, in general, except when specifically indicated in the results, were no more common in the experimental larvae than in the controls. There is no indication that in eggs centrifuged before fertilization and in some experiments following fertilization that there is any differential mortality or aberration.

In those counts that were made to determine whether the centrifuging influenced the bilateral axis, only those embryos were counted that had the centripetal oil in the prototroch or trochophore cilia band. Similarly, to determine whether there was any influence on the polar axis, counts were made of trochophores that had the oil in the region anterior to the prototroch derived normally from the animal hemisphere and those with the oil in the post-trochal and gut regions normally derived from the vegetative hemisphere. The first hundred embryos seen in any experiment were grouped into types and the number found of any type is therefore the percentage for that experiment based on a random count. Any exceptions are specifically explained in the results.

Results Concerning the Bilateral Determination

Different forces were applied for different lengths of time in ten experiments on the unfertilized eggs. Directly after centrifuging the eggs were fertilized. The data obtained are summarized in Table I. The categories in which the trochophores were grouped are, of necessity, somewhat arbitrary. Only trochophores with the oil present in the prototroch are recorded. In the first group it was found in the nearly median plane on the ventral side. In the second group it was ventral oblique, in the third nearly lateral, and in the fourth group dorsal or dorsal oblique.

As will be seen in Table I, of a thousand embryos counted at random and grouped into these categories, 41.1 per cent had the centripetal oil in the ventral median position, 33.2 per cent had the oil in the ventral oblique position, 21.5 per cent had it in a lateral position, and only 4.2 per cent had the oil dorsal or dorsal oblique. It is quite clear from a study of the table that the centrifugal force applied over a range of from 6,000 to more than 75,000 gravities makes no significant difference in the relative number of embryos found in any particular group. The lowest force used gave essentially the same result as the greatest force.

Table I

Eggs centrifuged before fertilization tabulated to show the force applied and the number of embryos recorded with the centripetal oil in the various possible positions in relation to the dorso-ventral axis.

Experiment Number	Force in Gravities	Time in Minutes	Oil Ventral Median	Oil Ventral Oblique	Oil Lateral	Oil Dorsal
1	6,000	3	40	35	21	4
2	6,000	6	38	34	26	2
3	6,000	12	39	30	26	5
4	20,000	3	45	35	17	3
5	50,000	3	44	36	17	3
6	50,000	5	41	36	16	7
7	50,000	12	41	35	22	2
8	75,000	5	37	31	28	4
9	75,000	5	34	33	20	5
10	77,500	3	44	27	22	7
Total per cent			41.1	33.2	21.5	4.2

It may be said that experiments Nos. 8, 9, and 10 represent the greatest forces that could be applied without bursting almost all of the eggs.

Table II tabulates similar data on eggs centrifuged at various times after fertilization and with various forces. The complete summarized data on the development and stage of centrifuging of these eggs appears tabulated in Table IV under the same experiment numbers. The percentage of the total with the oil in the ventral position is 44 per cent, with the oil ventral oblique 33 per cent, with it lateral 19 per cent. Only 4.5 per cent had the oil in the dorsal region.

It is apparent that there is no significant difference in the results depending upon the time after fertilization at which the eggs were centrifuged or between eggs centrifuged before and after fertilization. This work includes experiments carried out during the breakdown of the germinal vesicle, after both the first and second polar body formation, and just before cleavage. The influence of the centrifuging on the determination of the dorso-ventral axis is the same irrespective of the centrifugal force used.

In order to preclude the possibility that there might be some bilateral orientation in the centrifuge tubes, eggs were centrifuged in stiff gelatin before fertilization. A solution of gelatin in sea water was made that with rapid cooling remained liquid until 16° C. was reached, at which temperature the solution jelled. The eggs were put in the solution at 22° in the centrifuge tubes and cooled until the gel was quite stiff and held the eggs very firmly. The eggs were centrifuged for five minutes at 50,000 gravities, removed from the gel by breaking it up and

TABLE II

Eggs centrifuged after fertilization. The complete data on the times after fertilization at which the eggs of these experiments were centrifuged are included in Table IV under the same experiment numbers.

Experiment Number	Force in Gravities	Time in Minutes	Oil Ventral Median	Oil Ventral Oblique	Oil Lateral	Oil Dorsal
12	50,000	5	45	39	15	1
13	6,000	5	41	36	17	6
14	50,000	5	42	33	16	9
16	50,000	5	44	27	22	7
17	20,000	5	48	29	20	3
20	50,000	3	44	32	23	1
Total			264	196	113	27
Total per cent			44	33	19	4.5

dissolving it in sea water at 22°, and then fertilized. The position of the oil in the trochophores was recorded in two hundred embryos. In 45 per cent the oil was ventral median, in 34 per cent ventral oblique, in 19 per cent it was lateral, and in only 2 per cent was it found dorsal. These figures correspond to the results obtained when the eggs were centrifuged in a free suspension of sugar and sea water and it is concluded that there is no orientation of the eggs in the centrifuge tubes in relation to the presumptive bilateral axis.

The influence of the centrifugal force on the determination of the bilateral axis is concluded to be the same in all experiments irrespective of the time of centrifuging before cleavage, or the force applied above 6,000 gravities. It has already been indicated in the previous section that there is no indication of a differential mortality. The centrifugal force has either induced a bilateral axis or shifted the presumptive axis. There is no evidence that a presumptive axis remains fixed and that the mortality of certain types is higher than others.

Position of the Cleavage Planes in Centrifuged Eggs

In the very large majority of eggs, centrifuged before or after fertilization, the first cleavage plane passed through the axis of centrifuging and the pole as marked by the polar bodies. In a small percentage it passed through the pole and oblique to the axis of centrifuging. In an even smaller percentage it passed through the pole and at right angles to the axis of centrifuging. The second cleavage also passed through the pole and was at right angles to the first cleavage.

It has been found by Just (1912), Morgan and Tyler (1930), Tyler (1931), and Tayler (1931) that the first cleavage has a tendency to pass through the sperm entrance point in spirally cleaving eggs. Since the first cleavage in spirally cleaving eggs bears a definite relation to the *4d* cell, and hence to the plane of bilateral symmetry, it is important to consider whether the first cleavage plane is related to the sperm entrance point in centrifuged eggs. The fertilization process of a large number of eggs that had been centrifuged was watched and the sperm was seen to enter any part of the egg and to show no preference for any particular region. Such a relation is therefore unlikely since in the great majority of eggs the first cleavage coincides with the axis of centrifuging and passes through the pole. It is statistically obvious that in most cases it cannot also pass through the sperm entrance point.

The question also arises as to whether the first cleavage plane is directly related to the dorso-ventral axis of the embryo since a small percentage of the eggs do cleave obliquely and at right angles to the axis of centrifuging. To examine this question a number of eggs were isolated in the two- and four-cell stage according to their cleavage pattern in relation to the axis of centrifuging. These eggs were centrifuged at 20,000 gravities for five minutes before they were fertilized. The results are tabulated in Table III. The number of embryos recorded is too small for statistical study, but it is quite clear that any one type of cleavage may produce trochophores with the oil in all common positions. It is concluded that in the centrifuged eggs the cleavage plane is not directly related to the bilateral axis of the embryo.

Results Concerning Development

It has already been indicated that there is normal development in the majority of eggs centrifuged before fertilization. However, that is distinctly not the case in some of the experiments on eggs centrifuged after fertilization. The developmental history of various experiments is briefly indicated in Table IV as far as it was noted. Experiments Nos. 15, 18, and 19 produced too few normal trochophores to be of value in making statistical counts of the positions of the oil in the embryos. Experiments Nos. 14 and 16 produced proportionately few normal embryos, but enough for statistical studies. In these experiments most

Table III

The position of the centripetal oil in relation to the bilateral axis of the trochophores is tabulated. The trochophores were reared from eggs isolated in the two- and four-cell stages according to their cleavage pattern in relation to the axis of stratification.

Trochophores	Two-cell stage		Four-cell stage		Total	Percentage
	First cleavage plane parallel to stratification	First cleavage plane at right angle to stratification	Oil evenly distributed in two of four cells	Oil in only one of four cells		
Oil ventral median.....	12	15	27	19	73	41
Oil ventral oblique.....	5	14	27	19	65	36
Oil lateral............	3	9	22	5	39	22
Oil dorsal.............	0	0	1	2	3	2
Total............	20	38	77	45	180	101

eggs cleaved irregularly and stopped development before good morulae were formed. Experiments Nos. 12, 13, 17, and 20 produced normal trochophores from the majority of eggs. Unfortunately there are no data on the time of first polar body extrusion in Experiment No. 13, but centrifuging was probably shortly before its formation.

In general the abnormal development of the eggs in some of the experiments, centrifuged after fertilization and before cleavage, seems to result from interference with the maturation and cleavage spindles as Morgan and Tyler (1935) have indicated. However, these authors got very little normal development in their experiments on eggs centrifuged after fertilization.

Table IV

The data on eggs centrifuged after fertilization are tabulated as far as recorded, including the time of centrifuging in relation to the polar body formation and cleavage, polar orientation observed to have been present by the position of the oil in the trochophores, and the general viability.

Experiment Number	Time after Fertilization of Germinal Vesicle Breakdown	Time of First Polar Body Formation	Time of Second Polar Body Formation	Time of Cleavage	Time of Centrifuging after Fertilization	Centrifugal Force Applied in Thousands of Gravities and Minutes	Cleavage and Development
12	15			85	15	50Gs. 5M.	Normal development, no polar orientation since the oil is commonly found in both the anterior and post-trochal regions of the trochophores.
13				85	28	6 5	Normal development, polar orientation since the oil is commonly found only in the post-trochal regions of the trochophores.
14		30	45	85	32	50 5	Few normal trochophores developed, polar orientation.
15		40	55	90	45	20 5	Delayed and irregular cleavage, second polar body suppressed, first cleavage often into three cells. Very few survived normally.
16		30	45	85	47	50 5	Few normal trochophores developed, polar orientation.
17		40	55	90	60	20 5	Cleavage regular, normal development, polar orientation, a few exogastrulae.
18				90	70	20 5	Delayed and irregular cleavage, very few survived normally.
19		40	55	90	80	6 5	Delayed and irregular cleavage, very few survived normally, a few exogastrulae.
20				90	82	50 3	Normal development, polar orientation.

Results Concerning Polar Orientation

It was observed in the trochophores of eggs centrifuged after polar body extrusion that the centripetal oil was almost invariably found in the post-trochal region, in the gut extending not much further towards the animal pole than the prototroch. This suggested that there might be a polar orientation of the eggs in the centrifuge tubes, a question that has been examined in part by Morgan and Tyler (1935) using lower centrifugal forces. In the present case the position of the polar bodies were determined in eggs which were centrifuged at various times

Table V

The position of the polar bodies in eggs centrifuged at various times after fertilization is tabulated. The complete data on the eggs and development are included in Table IV under the same experiment numbers. The bracketed numbers express the total figures in terms of percentage for the particular experiment.

Experiment Number	Time of Centrifuging (Minutes after Fertilization)	Centrifugal Force	Polar Bodies					
			Oil Cap	Oil Cap to Equator	Equator	Equator to Third Layer	Boundary Second and Third Layers	Centrifugal Third Layer
11	Before fertilization	20,000 5 min.	21	23	—	18	—	38
17	60	20,000 5 min.	0 (0)	5 (3)	12 (8)	24 (16)	29 (19)	79 (53)
18	70	20,000 5 min.	4 (1)	48 (17)	33 (12)	62 (23)	52 (19)	75 (27)
19	80	6,000 5 min.	0 (0)	8 (9)	6 (7)	21 (24)	22 (25)	31 (35)
20	82	50,000 3 min.	0 (0)	24 (12)	29 (15)	63 (32)	49 (25)	35 (18)

after polar body extrusion. The number of eggs found with the polar bodies in these various positions is tabulated in Table V. Also a control experiment is included in which the eggs were centrifuged before fertilization.

For accuracy of counting only those eggs with polar bodies on or near the periphery as it appeared in the field under the microscope were counted. Temporary glycerine preparations were made and the whole slide scanned by means of a mechanical stage. Six groupings were made of the fertilized eggs, dividing the circumference of the egg into about five equal arcs and the slightly larger oil cap region.

In the group centrifuged before fertilization 44 per cent of the recorded eggs had the polar bodies above the equator in the centripetal end, and 56 per cent showed the polar bodies below the equator towards the centrifugal end. This shows little or no orientation of the pole in centrifuged unfertilized eggs and is in agreement with the results of Morgan and Tyler (1935) and Taylor (1931).

The four experiments in which eggs were centrifuged at various times after polar body extrusion all gave evidence of polar orientation on the centrifuge. This orientation seems to be rather variable at different times of centrifuging. Thus, in Experiment No. 17, centrifuged five minutes after the second polar body extrusion, only 3 per cent were found with the polar bodies above the equator, or 11 per cent including the equator, and 88 per cent had the polar bodies in the centrifugal hemisphere. On the other hand, ten minutes later (Experiment No. 18) 30 per cent had the polar bodies on or above the equator. Experiment No. 19, 10 minutes later, showed only 16 per cent with the polar bodies on or above the equator. Two minutes later, Experiment No. 20, 28 per cent were found with the polar bodies on or above the equator. A peculiar result of the last experiment is that practically no eggs had the polar bodies at the extreme centrifugal end, but the 18 per cent recorded from the centrifugal third and fourth layers in general had the polar bodies near the junction of the second layer.

Table VI

The position of the oil in relation to the polar axis in trochophores reared from eggs centrifuged before fertilization at various forces is tabulated and the results expressed in terms of percentage.

Experiment Number	Force in Gravities	Time in Minutes	Oil Ectodermal in Animal Hemisphere Anterior to Prototroch	Oil Endodermal in the Gut from the Vegetative Hemisphere
2	6,000	6	52	48
5	50,000	3	44	56
9	75,000	5	51	49
Total			147	153
Percentage of total			49	51

The position of the centripetal oil in relation to the antero-posterior axis of the trochophores is given in Table VI in which different centrifugal forces were used on unfertilized eggs. The oil was found in regions derived from the animal hemisphere in 49 per cent of the recorded embryos, and in the vegetative post-trochal regions in 51 per cent. The centrifugal force used made no significant difference. This result is in harmony with the result obtained counting the positions of polar bodies in eggs centrifuged before fertilization and in such eggs there is no indication of polar orientation in the centrifuge tubes.

Rough observations were made on the position of the oil in the trochophores from eggs centrifuged after fertilization. In Experiments Nos. 17 and 20, in which polar body counts had demonstrated that there was an orientation in the centrifuge tubes, the oil in the trochophores was most commonly found in the post-trochal region and rarely at the anterior end. In Experiments Nos. 13 and 14, centrifuged just before and just after the first polar body extrusion, trochophores were found to have the oil in general in the post-trochal region, indicating that these eggs also oriented in the centrifuge tubes. On the other hand, Experiment No. 12, centrifuged at the time of the breakdown of the germinal vesicle, indicated no polar orientation as the oil in the trochophores was found commonly in all regions of the embryo.

The general conclusions to be drawn from these results are that the eggs of *Urechis* do not orient in the centrifuge tubes before fertilization or following fertilization until after the breakdown of the germinal vesicle. Before the first polar body extrusion and until cleavage there is a greater or lesser tendency for the eggs to orient, in general with the pole near the centrifugal end.

Morgan and Tyler (1935) find no polar orientation in unfertilized eggs. In fertilized eggs centrifuged after the germinal vesicle breakdown and before the first polar body extrusion they find a decided tendency for the polar bodies to be extruded near the centrifugal end. They point out that this may mean a shifting of the pole rather than orientation. In three experiments centrifuging eggs after the first polar body extrusion, and one after the second polar body extrusion (data combined in their Fig. 3), they found a random distribution of the polar bodies, with no indication of orientation in the centrifuge tubes. Their method was that of centrifuging eggs in sea water in an electric centrifuge developing about 2,000 gravities. The packing effect of the eggs in the bottom of the tubes may have prevented a free rotation of the eggs, or the discrepancy between their results and mine may be due to some other more subtle reason. It is possible that at certain miotic stages there is no tendency for the eggs to orient.

These authors have also shown that in eggs centrifuged before the second polar body extrusion the second polar spindle may be driven far from the pole. In my Experiment No. 15, centrifuged five minutes after the first polar body extrusion, second polar body formation was suppressed, and the first cleavage was in general into three cells, as might be expected (Tyler and Bauer, 1937) if the spindle was driven into the interior of the egg.

Conclusions

The results of centrifuging unfertilized and fertilized eggs are essentially the same in regard to the position of the plane of bilateral symmetry in relation to the stratification. Further, there is no significant difference between experiments in which widely different centrifugal forces were used, or in which the eggs were centrifuged at widely different times after fertilization.

In 75 per cent of the total number of trochophores recorded the centripetal oil was found in the ventral or ventral oblique position. In slightly more than 20 per cent it was in a lateral position, and in less than 5 per cent it was found in the dorsal or dorsal oblique position. There can be no doubt, I think, but that *the centrifuging induces or influences the bilateral or dorso-ventral axis in the egg.* This induction is probably by the establishment of material gradients, substances concentrated in a fairly wide stratification layer at the centripetal end which influence the direction of gastrulation and determine the ventral side.

In the early work in centrifuging spirally cleaving eggs (Boveri, 1910; Conklin, 1910 and 1916; Hogue, 1910; Lillie, 1909*a* and *b;* Morgan, 1910; von Parseval, 1922; Schleip, 1914; Spooner, 1911; Tyler, 1930; Whitney, 1909; and Wilson, 1929 and 1930) embryos were obtained in some cases with the centripetal oil in any position. However, no counts were made to determine the relative numbers with the oil in the different regions, and the centrifugal forces used may well have been too small to have had any effect upon the determination of the bilateral axis. Morgan (1910), who has given the most data on this point for the egg of *Cumingia,* used a hand-turned centrifuge that developed a force of about 800 gravities. Conklin (1910) studied the effects of centrifugal forces of 600 gravities on various freshwater pulmonate eggs (*Physa* and *Lymnea*) and reported no influence on the bilateral determination. The development of the mosaic eggs of ascidians following low speed centrifuging has been studied with great care by Conklin (1931). He has found no evidence that the bilateral axis as a whole is in any way shifted by the forces used although different "organ-forming" substances have been displaced causing abnormal development.

The echinoderm egg, on the other hand, through the work of Runnström (1926) and Lindahl (1932), has been shown to develop with the bilateral axis of the pluteus definitely related to the stratification of the egg. My results (in press) of an analysis of *Dendraster* embryos developing from ultracentrifuged eggs have indicated that a predisposed dorso-ventral axis is shifted by the centrifugal forces. This analysis is based upon the fact that the amount of axial rotation induced by the centrifugal force is proportional to the force applied and is at best only a partial effect—the greater the force applied, the greater the percentage of plutei with the centripetal end in the median ventral plane, and the smaller the percentage with the centripetal end lateral. It was concluded that two factors must be involved in the bilateral determination, one factor present in the egg as a gradient and unshifted by the centrifugal force, and the other, probably diffusely distributed in the endoplasm, which is stratified towards the centripetal end to a greater or lesser extent with different forces. The plane of symmetry is then determined at the region of the greatest interactivity btween these two factors.

These results are in striking contrast with those obtained for *Urechis* eggs in which no significant difference was obtained in the relative percentages of the various described types when widely different centrifugal forces were used. It is therefore impossible to apply the type of analysis to these *Urechis* experiments that I have applied to *Dendraster*.

Because the *Urechis* experiments have given less pertinent data than the *Dendraster* experiments, at this time it seems inadvisable and impossible to attempt analysis of the factors involved in the dorso-ventral determination. There are, however, certain things that may be said.

In normal development Tyler (1931) and Tayler (1931) have shown that the sperm entrance point in general coincides with the first cleavage in *Urechis*. Assuming development as in *Thalassema* (Torrey, 1903) the first cleavage may very well then be related to the bilateral axis in normal development. However, Hörstadius (1937) has shown that in *Cerebratulus* the first cleavage may bear any relation to the bilateral axis. I have attempted to test this point in *Urechis* by vitally staining one blastomere but so far all the vital stains I have used have proved toxic.

Tyler (1931) has shown that normal trochophores may be obtained from artificially activated eggs of *Urechis*. In these experiments the sperm entrance point can play no part in the bilateral determination. In the centrifuged eggs I have already pointed out that in the majority the first cleavage cannot pass through the sperm entrance point. Nor can the sperm entrance point directly establish in these eggs the bilateral symmetry since it is random but the bilateral axis is related to the axis of centrifuging. Isolation experiments, rearing separately various cleavage types in relation to the axis of centrifuging, show that the first cleavage in these eggs is also unrelated to the determination of the dorso-ventral axis.

Hörstadius (1937), following the work of Yatsu (1910), has been unable to show any indication that the two- or four-cell stage of *Cerebratulus* possesses a bilateral predisposition. He studied the development of isolated half- and quarter-blastomeres and attempted unsuccessfully to apply the type of analysis that Hörstadius and Wolsky (1936) were able to use to demonstrate the presence of a bilateral predisposition in the early cleavage stages of *Paracentrotus*. His conclusions are that in *Cerebratulus* no apparent bilateral determination exists in the early cleavage stages.

Lillie (1909) and Wilson (1929 and 1930) have studied the development of partial eggs of *Chaetopterus* fragmented by centrifugal force. The first cleavage is found to be unequal and Wilson finds that in general it is at right angles to the stratification. Lillie concludes from the fact that the early cleavages closely resemble the normal that the bilateral axis in the uncleaved egg is present in the "ground substance" and is undisturbed by the fragmenting except in so far as an epigenetic adjustment is made regulating the size of the blastomeres to approximate the normal proportions. From such fragments Wilson has obtained quite normal dwarf larvae. He also has observed that the cleavage is dextriotropic as normal. However, since these eggs orient in the centrifuge tubes so that the polar axis comes to coincide with the axis of centrifuging, Lillie's conclusion that the bilateral axis is fixed in the egg and undisturbed by the centrifugal force seems quite unwarranted. The effect of centrifugal force on the bilateral determination of these eggs can only be studied if the eggs are held so that the polar axis is more or less at right angles to the axis of centrifuging.

It is not yet possible to say definitely that there is no bilateral predisposition in the unfertilized or fertilized egg of *Urechis*. These experiments show only that in centrifuged eggs the bilateral axis is unrelated directly to the sperm entrance point and the first cleavage plane, but is definitely influenced by the axis of stratification. It is quite clear from cell lineage studies that in spirally cleaving eggs the location of the ventral side is given by the position of the *4d*-cell and its derivatives. It seems most likely to suppose that the centrifuging induces a material gradient in the uncleaved egg that later functions to fix the properties of the *D*-cell at or near the centripetal end of the egg if the axis of centrifuging makes a considerable angle with the polar axis. It seems best to regard this bilateral determination as induced in the egg and not as a rotation or shift of a bilateral axis or predisposition already present in the egg, although such may be present in a weakly defined form.

The experiments of Hörstadius (1937) and Wilson (1929) have been particularly significant in showing that egg fragments of spirally cleaving eggs may produce dwarf larvae that seem otherwise normal. Such development cannot be called strictly "mosaic." In these experiments the dorso-ventral axis has been determined by a stratification of material substances in the *Urechis* egg. Such a modification of the normal growth pattern is also not in harmony with a strictly determinate theory of development. Bilateral determination in this egg must be regarded as indeterminant or at least quite labile.

Summary

1. The eggs of *Urechis caupo* were ultracentrifuged for short periods with forces ranging from 6,000 to 75,000 gravities before fertilization and following fertilization at various times before cleavage.

2. In the trochophores 75 per cent of the total recorded embryos had the centripetal oil in the ventral median or oblique position, in slightly more than 20 per cent it was found laterally, and in less than 5 per cent dorsally.

3. The data are essentially the same for unfertilized eggs and all eggs centrifuged at various times following fertilization and before cleavage, and irrespective of the force applied.

4. From these data it is concluded that the bilateral axis of the embryo is induced or influenced by the stratification of material substances in the egg. In centrifuged eggs it is unrelated to either the sperm entrance point or the first cleavage plane.

5. There is no polar orientation in the centrifuge tubes before fertilization or following fertilization until the germinal vesicle breakdown. But following the germinal vesicle breakdown the eggs tend to orient in the centrifuge tubes so that the pole comes to lie most commonly near the centrifugal end.

6. Normal trochophores are produced from eggs centrifuged before fertilization and at certain times following fertilization, but during some miotic and mitotic stages centrifuging suppresses polar body formation and disturbs the cleavage spindles so that few normal embryos are produced.

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